November 6, 2009

Carbohydrate Clustering to Bio-sensing Application

My motivations for getting involved: Growing up in Thailand in a city called Chiangmai, I always thought I would either end up being a doctor, a number one job that is most respected in my society. As much as I knew that studying medicine was most likely to provide me and my family good living condition in the future, I was not excited about this prospect. Upon my graduation from high school, I applied and won a Royal Thai scholarship, which introduced me to nanotechnology. The fact that matter could be manipulated on an atomic level aroused my energy and curiosity and inspired me to pursue a higher education and career in nanotechnology. I was not aware then that the subject could be so broad. So I chose to focus in bioengineering during my first year at the University of Washington hoping to be able to contribute to science as well as improve the quality of lives.

Through collaboration between Professor Daniel Ratner, my advisor in the bioengineering department and Dr. Dirk Weiss, my advisor during my summer internship at Washington Technology Center (WTC), I had an opportunity to get hands-on experiences in applying nanotechnology to help study bioengineering problems last summer. I enjoyed learning new skills during that period and now challenge myself to push the complexity of the project to a higher level by adding more biological meanings into it. Therefore, this ongoing study is integrated into my senior capstone project, an individual design project required in the bioengineering department, which I plan to dedicate my time to throughout this academic year under the supervision of Prof. Ratner and Dr. Weiss.

Description of my research to date: The overall theme of my independent research project is the study of mixed carbohydrate-functionalized self-assembled monolayers (SAMs) on gold surfaces. SAMs have become an important method to modify the properties of and to add functionality to surfaces with a variety of applications, such as bio-sensors and electronic devices. A previous study in the Ratner lab has shown that the molar ratio of carbohydrates in a mixed SAM (consisting of functional carbohydrates and non-functional molecules) affects the protein binding capacity on the surface [1]. We therefore revisited the common assumption that mixed SAMs grow with uniform distribution. Using a mixed-SAM model system: synthetic tetrasaccharide and oligo(ethylene glycol) moieties, we wanted to obtain a better understanding of SAMs formation on the molecular level. This research, which I am carrying out during the summer and fall of 2009, suggests that carbohydrates form clusters, resulting in a phase-separated SAM. We hypothesize that the strong hydrogen bonds between carbohydrates cause the observed aggregation. Using atomic force microscopy (AFM)—a characterizing technique capable of imaging surface topography with nanometer resolution—I was able to show that this clustering phenomenon of glycans occurs as a function of time (Figure 1).

Description of my proposed research: Observing the phenomenon, I then want to evaluate the effect of clustered functional SAM to protein binding applications. The hypothesis is that a protein is likely to bind to the glycan molecules at the edge of the clusters because those molecules at the edge are not as tightly packed as ones in the middle of the clusters; and are therefore less hindered for proteins to grab onto. From a preliminary data of AFM images, it is difficult to determine an exact location of protein binding to naturally clustering glycans because the pattern is irregular. Consequently, I propose to design a glycan platform that will have well

defined SAMs structures so that a specific protein binding is clearly recognizable in AFM. Such a platform will be made by patterning artificial arrays of SAM-patterns using dip-pen nanolithography (DPN) in the WTC's Microfabrication Laboratory. DPN is a lithography technique that allows precise construction of nanostructures of SAMs.

Specifically, the system will include SAM functionalized with the polysaccharide β(1-3)-*N*-acetyl galactosamine (GalNAc) and cyst wall proteins (CWPs) that bind to it. While this system will allow me to study the binding location of proteins on glycan clusters, it can also be used to study the more in-depth interactions between GalNAc and CWPs, the two known components of Giardia cyst wall. Giardia is an intestinal protist that can cause chronic diarrhea disease in a host. Briefly, a study by Prof. Ratner's collaborators suggests that CWPs consist of two domains and each domain may have different affinities toward GalNAc molecules. We are interested in the Giardia cyst wall because this cyst wall material is uniquely strong. It protects the organism in its transmitting form from severe environments, such as bacterial degradation and the host immune system. By constructing this platform and using it to characterize the material, we hope to eventually develop prophylactics and potential therapies for diseases caused by Giardia infection. Additionally, we hope that the knowledge will enable us to reverse engineer the cyst wall material. Being natural, bacterial resistant, and specific to degradation, the mimicked cyst wall may also be useful in other medical applications, such as drug delivery and implants.

The contributions of this project to my education and others and to the development of my future plans: While I will be able to advance the skills I have previously learned and also acquire new set of skills such as constructing the platform using DPN and processing images for data analysis, this project will benefit me in non-technical aspects as well. The opportunities to

interact with more academics and experts in my field of study will educate and broaden my visions while establishing connections that may be useful in my future. My communication skill will be pushed to a higher level through the writing of reports and papers, the presentations in weekly group meetings and discussions on the project with Prof. Ratner and Dr. Weiss. Moreover, I feel that I am becoming more patient as the complexity of our study amplifies and troubleshooting is constantly required. I have grown more confident and ambitious about my future and I believe that those qualities will continue to flourish much more through my experiences from this project.

As much as I am learning from this project, I hope that my work also benefits my colleagues. To the Ratner lab, where the surface plasmon resonance imaging is a primary technique used to study the protein bindings to carbohydrate-functionalized surface, I hope to contribute the understanding of the binding events from a different perspective through AFM study. The work on Giardia cyst wall can provide new insights to other researchers who also study this material. Upon the completion, this project may also initiate more applications of DPN technique which is currently limited.

After I graduate, I intend to carry on my passion in bionanotechnology by pursuing a Ph.D. in bioengineering in the U.S. I hope to go back to my country and inspire the love of science and research in others as a professor in a university. I am determined to strengthen bioengineering and nanotechnology research in Thailand, and I dream about taking part in improving Thai education. The Mary Gates Research Scholarship will fuel this project by allowing me to work at my full capacity. Consequently, through the scholarship, I will be able to acquire the knowledge and skills that will enable me to reach out and give back to my community.

Reference

[1] Marshal Dhayal, Daniel M. Ratner. (2009). XPS and SPR Analysis of Glycoarray Surface Density. *Langmuir 25* (4), 2181-2187.

Terminology

Atomic Force Microscopy (AFM): A technique that visualizes surfaces by "feeling" to produce the topograph of the sample surface. It measures the angle change of light that is reflected on cantilever with the AFM tip tapping on the surface (for tapping mode). The angle changes correspond to the changes of vertical height of the surface in nano scale.

Cyst: A life form of some organism where the cells are shielded by an outer self-produced shell.

Dip-Pen Nanolithography (DPN): A technique that uses AFM tip (radius in nanometers) to deposit materials onto a surface in any desired pattern. It is analogous to drawing using a brush and an ink.

Giardia: A eukaryote that is nearest to Bacteria and Achaea groups, and furthest to humans. The infection can cause chronic diarrhea disease called Giardiasis.

Glycan: A polysaccharide.

Glycoarray: An array platform of carbohydrates with high throughput and high sensitivity commonly used to study molecular interactions between glycans and proteins.

Nanotechnology: A study of matter in 10^{-9} meter scale.

Protist: A group of organisms with simple organizations (single cells or multi cells that do not form a particular tissue.)

Self-Assembled Monolayer (SAM): An organized layer of molecules consisting of head groups that attract to a substrate and tail groups that become surface of the substrate.

Surface Plasmon Resonance Imaging: A detection technique that provides a real-time association and dissociation of molecules in term of mass concentration on the surface.

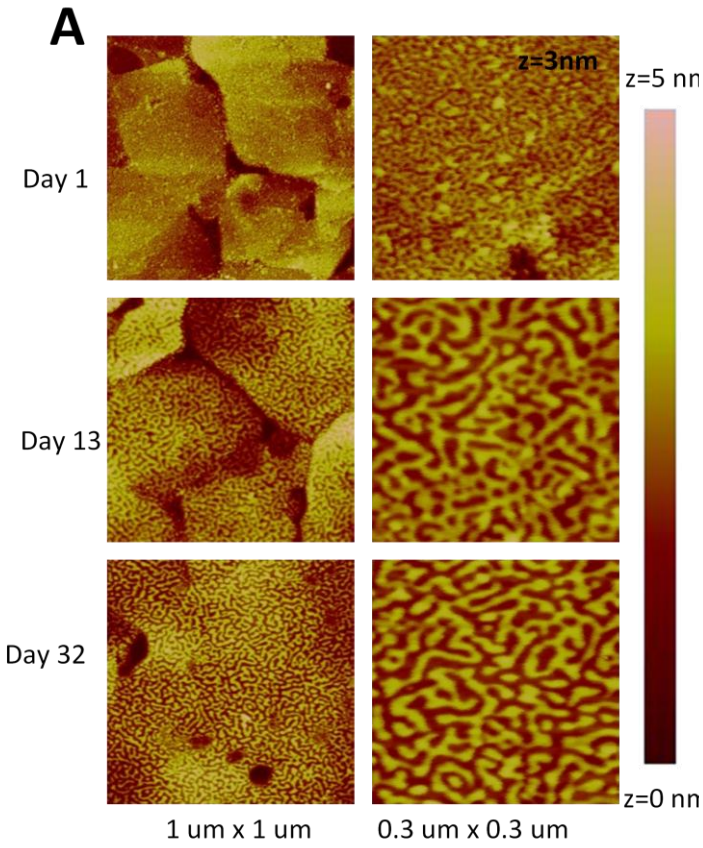

Figure 1 AFM height images showing clustering phenomenon of tetrasaccharides over time.